and has poor transplant outcome with a high TRM, a lower EFS and a lower OS.

3. Group 2 (0.30- 0.85 $\times$ 10^8/kg) had the best overall engraftment (both ANC and PLT) of close to 80%. TRM was lowest in this group at 13% but the rate of relapse was higher than the other groups.

4. Group 3 (0.86- 2.83 $\times$ 10^8/kg) received higher than the median MNC cell dose. Compared to Group 2 though this group shows a lower engraftment it shows comparable EFS. This group shows that a higher cell dose results in higher TRM (8 of 9 patients) and fewer relapse (4%).

Transplant Outcome

MNC dose group ($\times 10^8$/kg	Overall (0.08- 2.83)	(1) ≤0.29	(2) 0.30- 0.85	(3) ≥ 0.86
n=	92	19	46	27
ANC engraftment	63; 68.5%	10; 52.6%	38; 79.2%	15; 55.6%
PLT engraftment	58; 63%	10; 52.6%	36; 78.3%	12; 44.4%
Days to ANC	24	36	26	18
Days to PLT	44	56	46	34
TRM	22; 23.9%	8; 42%	6; 13%	8; 29.6%
Relapse	13; 14.1%	3; 15.8%	9; 19.6%	1; 3.7%
EFS	57; 62%	8; 34.8%	31; 67.4%	18; 66.7%
OS (days)	706	326	1396	603

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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL DISEASES AT YEDITEPE UNIVERSITY HOSPITAL

Yirmibescik, S.[1], Senkal, V.[1], Kalayci, G.[1], Aktas, S.[1], Aydin, D.[1], Sonmezoglu, M.[1], Kocagoz, S.[1], Oyan, B.[1], Koc, Y.[1] [1]Stem Cell Transplant Unit, Yeditepe University Hospital, Istanbul, Turkey.

Stem cell transplantation(SCT) is an effective treatment modality in hematological malignancies. SCT Unit at Yeditepe University Hospital(CIC-919) was activated in October 2005 and accredited for unrelated SCT by EBMT in April 2006. Until September 2006, 13 autologous and 17 allogeneic (4 unrelated) SCT have been performed. Diagnosis of patients who underwent auto-SCT consisted of HL(n=6), NHL(n=4) and myeloma (MM, n=3). Patients undergoing allo-SCT had HL(n=2), NHL(n=2), MM(n=3), CML(n=3), CML-BT(n=3), AML(n=2), ALL(n=1), and Thalassemia(n=1). Median age was 38,5 (20-65) years and mean time from diagnosis to transplant was 2,3 years. Patients received a mean number of 8 (2-18) salvage regimens prior to transplantation. All patients engrafted and median engraftment period was 11 (8-14) days. Transplantation related mortality(TRM) was not observed during the first 100 days and follow-up. Median follow-up period was 5,4 (1-10,5) months. During follow-up, 5 patients (16%) relapsed and 2 patients died (6%). Of 28 (94%) surviving patients, 24 (80%) are in CR. All patients (100%) were alive following auto-SCT at day +100, only one patient with myeloma died due to relapse at 9 months. In the allo-SCT group, 16 (94%) of patients were alive at day +100, only one patient died due to relapse of CML-BT. Post-transplant complications were CMV viremia(n=9), CMV colitis(n=3), sinusoidal occlusion syndrome(n=1), BK viremia and hemorrhagic cystitis(n=2), renal failure(n=1). Reversible blindness due to hypophyseal tumor apoplexy in a patient at day +11 was successfully corrected by neurosurgery. Grade II- III acute GVHD was observed in 8 patients(47%). All patients with acute GVHD were successfully treated without mortality. A patient with refractory ALL associated with CNS and eye involvement achieved complete remission following allo-SCT combined with modulated radiotherapy (IMRT). All 4 patients (100%) who underwent unrelated-SCT following autologous transplantation complicated with relapsed-refractory disease are alive and 3 were in CR at day +100 (2 HL,1 NHL). Of 7 patients undergoing allo-SCT as a second transplant, 6 (85%) are alive; in this group, only one patient with MM (15%) died due to relapse. The high survival rate (94%) achieved following autologous and/or allogeneic SCT including unrelated transplants, low TRM (0%) and the high remission rate (80%) may be related to team approach, 24-hour patient follow-up, and effective management of acute GVHD.

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VALIDATION OF THE HEMATOPOIETIC CELL TRANSPLANTATION-COMORBIDITY INDEX (HCT-CI) FOR NON-RELAPSE MORTALITY (NRM) AND SURVIVAL AFTER MATCHED UNRELATED DONOR SCT

Kovacsovics, T.[1,2], Hayes-Lattin, B.[1,2], Riegert, K.[1], Subbiah, N.[1], Curtin, P.T.[1,2], Leis, J.F.[1,2], Mauro, M.M.[1,2], Epner, E.E.[1,2], Fleming, W.H.[1,2], Meyers, G.M.[1,2], Deininger, M.W.[1,2], Dunn, A.[1], Maziarz, R.T.[1,2] [1]Center for Hematological Malignancies, OHSU, Portland, OR; [2]Oregon Cancer Institute, Portland, OR.

Background: The HCT-CI is a recently developed comorbidity score which has been adapted to hematopoietic stem cell transplantation, with higher scores associated to worse outcomes (Blood 2005;106:2912) . We determined the HCT-CI score in a cohort of patients who underwent conventional MUD transplantation in a single arm, single institution trial assessing efficacy of a 3-drug combination of cyclosporine, methotrexate and prednisone for GVHD prophylaxis from 1996-2005.

Methods: The analysis included all patients undergoing MUD transplant who received GVHD prophylaxis with cyclosporine 2 mg/kg iv BID from day -2, methotrexate 15 mg/m2 iv on day +1 and 10 mg/m2 iv on days +3 and +6, and methylprednisolone 0.25 mg/kg iv BID beginning on day +7 and tapering at day +28. Patients were stratified by disease risk per CIBMTR classification. The comorbidities were obtained by retrospective chart review and scored according to the HCT-CI score.

Results: 133 patients received the 3 drug-regimen, including 26 % with low-, 36 % with intermediate- and 38 % with high-risk disease. Diagnoses included acute leukemia in 50%, MDS in 9.8%, CML in 16.5%, lymphoma in 18.1%, multiple myeloma in 3.0%. 52 % were older than 40. Source of stem cells was PBSC in 47.4%, marrow in 51.9%. and both in 0.8%. Among the 133 patients, 22%, 31% and 47% had HCT-CI scores of 0 vs 1-2 vs ≥3, respectively. Overall NRM was 26.3% and 36.8%, at 3 months and 1 year, respectively. Three and 12 month NRM was 13.3%, 14.6 and 40.3% and 30%, 22% and 50% among patients with scores of of 0 vs 1-2 vs >3, respectively. HR rates for 3 month NRM were 1.10 and 3.7 for HCT-CI scores 1-2 and ≥3. Kaplan-Meier assessment showed 42.1% 5 year OS for the whole cohort. OS of 60%, 48.% and 28.1% were observed in patients with scores of of 0 vs 1-2 vs ≥3, respectively ($p < 0.05$). No statistically significant differences in OS were observed between low-, intermediate- and high-risk CIBMTR disease groups.

Conclusion: HCT-CI was a powerful predictor of 3 and 12 month NRM, as well as of 5 year OS in this cohort of MUD patients. It will be useful for patient stratification in clinical trials and for treatment allocation.

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SYNGENEIC STEM CELL TRANSPLANTATION FOR APLASTIC ANEMIA IN AN HIV+ PATIENT

Longoni, H.O.[1], Binaghi, M.A.[1], Kohan, A.I.[1], Lasala, M.B.[1], Banchieri, A.V.[1], Minissale, C.J.[1], Plana, J.L.[1], Velicche, A.F.[1], Christin, M.P.[1], Corach, D.[2], Maissonnave, V.[1], Calahonra, R.[1] [1]Hospital de Clinicas José de San Martín; University of Buenos Aires, Buenos Aires, Argentina; [2]Facultad de Farmacia y Bioquímica; University of Buenos Aires, Buenos Aires, Argentina.

Allogeneic hematopoietic stem cell transplantation from HLA identical siblings is an effective treatment for aplastic anemia in young patients. Heavily transfused patiens have shown an increased risk of graft rejection requiring a more intensive immunosuppressive conditioning. Usually a combination of cyclophosphamide (Cy) with total body irradiation (TBI) or more recently with antithimocyte globuline (ATG) is used.

We report a 32 year old patient with haemorragic manifestations, haemodynamic instability and pancytopenia, admitted to the Hospital in February 2005. Bone marrow biopsy performed, confirmed the diagnosis of aplastic anemia, Human Immunodeficiency Virus (HIV) seropositivity was assessed by 2 different methods. No other

etiologic agent was found, so it was considered that AA was secondary to HIV. Treatment was initiated with D4T (Stavudine), 3TC (Lamivudine) and Efavirenz achieving undetectable viral load and increasing CD4 count. Patient also received Erythropoietin (EPO) and granulocyte-colony stimulating factors (G-CSF) showing an increased number of white blood cells (WBC) but continued with high transfusional requeirment. We had to stop treatment in October because of liver failure and lactic acidosis. In November we changed Stavudine for Tenofovir and reiniciated treatment. Viral load was always undetectable and CD4 count was > 500 cells/ul. However megakaryocytopoiesis and erythropoiesis did not respond, requiring many transfusions. Coombs Direct Test (CDT) and Coombs Indirec Test (CIT) were positive.He showed an immunohaematologic profile with 1 autoantibody (Anti-e) and 3 alloantibodies (Anti-Jka, Anti-Lua, Anti-Cw).

As he had an identical twin, he was submitted to a syngeneic BMT. Although he was heavily transfused (421 Units), we did not want to increase immunosuppression so as not to have viral reactivation. The conditioning regimen consisted of Cy 50 mg/kg/qd x 4, and in order to lower the risk of engrafment failure, peripheral blood stem cells were used to maximize the number of donor cells infused (11×10^6 CD34+ cells/kg). At the transplantation the patient was in high-risk. The antiretroviral treatment was not discontinued. No graft versus host disease (GVHD) profilaxis was needed. Neutrophils and platelets engrafted at day +11.

After 10 months of transplantation he continues in complete haematologic remission. Serum antibodies, CDT and CIT are negative.Viral load remains undetectable (b-DNA).

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ABSOLUTE CHIMERISM AS A TOOL IN MONITORING IMMINENT AND MANIFEST GRAFT REJECTION AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING

Masmas, T.N.[1], Petersen, S.L.[1], Madsen, H.O.[2], Ryder, L.P.[2], Svejgaard, A.[2], Andersen, P.[2], Dickmeiss, E.[2], Vindelov, L.L.[1] [1]Allogeneic Hematopoietic Cell Transplantation Laboratory, Department of Hematology, Rigshospitalet, Copenhagen, Denmark; [2]Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark.

Graft rejection after hematopoietic cell transplantation (HCT) with nonmyeloablative conditioning is a rare event and a serious clinical problem. Manifest (N=8) and imminent rejection (N=4) in a cohort of 98 consecutive patients with hematological malignancies were analyzed. The patients were conditioned with fludarabine 30 mg/m^2 and 2 Gy of total body irradiation and transplanted with peripheral blood stem cells. Intervention aiming at reversing imminent rejection with donor lymphocyte infusion (DLI) alone or preceded by immunosuppression with pentostatin was attempted with highly variable results. Chimerism analysis is the standard method to monitor engraftment and rejection. In the present report we have evaluated the product of absolute T cell counts and chimerism, which we have termed absolute chimerism, for monitoring patients with manifest or imminent rejection. The results suggest that recipient T cell counts $>$ donor T cell counts and increasing recipient T cells post-transplant are risk factors for rejection. Peaks of absolute recipient CD4+ and/or CD8+ T cell counts were seen in relation to rejection and peaks of donor CD4+ and/or CD8+ T cells were seen in connection with acute graft-versus-host disease. Furthermore absolute chimerism plots in some cases clearly indicate the time-interval where disappearance of recipient T cells takes place. These findings may be of importance for understanding the cellular mechanisms underlying alloreactivity. Absolute chimerism plots point to a variable immunosuppressive effect of pentostatin on recipient T cells as explanation for pentostatin/DLI-failure in reversing rejection. We conclude that absolute chimerism plots can contribute significant new information of value for routine monitoring of patients with mixed chimerism as well as for research purposes. Following rejection patients are at risk of dying from infections and progression/relapse of their malignancy. Retransplantation is feasible and well tolerated after HCT with nonmyeloablative conditioning. In patients with imminent graft rejection retransplantation is an attractive alternative to DLI or immunosuppression/DLI.

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AN IMPROVED METHOD FOR ENGRAFTMENT MONITORING USING REAL-TIME PCR

McLaughlin, I.J.[1], Bost, D.A.[1], Mountain, J.L.[2], McGinnis, M.D.[1], Krausa, P.[1] [1]Atria Genetics, South San Francisco, CA; [2]Department of Anthropological Sciences and Department of Genetics, Stanford University, Stanford, CA.

The commonly used engraftment monitoring method of short tandem repeat (STR) amplification following allogeneic hematopoietic stem cell transplantation (HSCT) is hampered by several shortcomings affecting sensitivity, accuracy, reproducibility, and results interpretation. In an effort to provide a better solution, we have developed a truly quantitative, real-time PCR method. Real-time PCR technology is ideally suited for this application because it is highly sensitive and quantitatively much more accurate and precise than STR analysis. It is not affected by problems inherent to STR amplification and analysis such as plateau bias, preferential allele amplification, and stutter artifacts. With our method, genomic mixtures are easily resolved, and analysis is straightforward and automatable. Success and maintenance of allogeneic HSCT may be enhanced with such an improved engraftment monitoring method.

We have developed a panel of non-repetitive polymorphic markers, with representatives on all chromosomes, useful for identifying differences between recipient and donor pairs. Our statistical analyses given allele frequencies in multiple human populations demonstrate that with relatively high minor allele frequencies, a limited panel of markers can be assembled which would have high probability of providing informative markers that could distinguish HLA-matched individuals. The testing process first requires that the donor and recipient genomic DNA be screened in order to identify informative markers between the two genomes. Once defined, these markers are used post-transplant to quantify the relative percentage of recipient genetic material in the donor background. Compared to the current STR assays, our method shows a greater than 100-fold increase in sensitivity with excellent accuracy and precision at concentrations as low as 0.01% recipient in a background of 99.99% donor DNA. Given the reproducibility of results and ease of data analysis, this approach provides the means for standardization within a testing lab as well as between testing centers, which cannot be accomplished with homebrew and off-label methods such as STR analysis. From a clinical perspective, improved sensitivity, accuracy, and precision provide the potential to detect disease relapse or transplant rejection at a much earlier stage. This information may be useful for improving decisions related to maintenance treatments such as GVHD prophylaxis and/or donor lymphocyte infusion.

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CHARACTERISTICS OF TWO CONDITIONING REGIMENS CYCLOPHOSPHAMIDE PLUS ANTITHYMOCYTE GLOBULIN VERSUS CYCLOPHOSPHAMIDE PLUS BUSULFAN IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

Ommati, L.V.M.[1,2], Silva, A.P.[1], Silva, L.P.[1,2], Faria, J.R.[2], Chaufaille, M.d.L.F.[1], Oliveira, J.S.P.[1,2] [1]Universidade Federal de São Paulo - UNIFESP, São Paulo, Brazil; [2]Hospital Santa Marcelina, São Paulo, Brazil.

We report a retrospective study of the clinical features and outcome for 32 allogeneic stem cell transplants (AlloSCT) from HLA-matched sibling donors in severe aplastic anemia conditioned with 200 mg/kg of cyclophosphamide plus 90 mg/kg of horse antithymocyte globulin (ATG) in 17 patients and 120 mg/kg of cyclophosphamide plus 12 mg/kg of busulfan (Bu) in 15 patients, at Hospital São Paulo and Hospital Santa Marcelina, from November 1993 to August 2003. Considering high cost of ATG and the difficulty to obtain it for the majority of our centers, the objective was to compare clinical variables as age, sex, engraftment, interval of time from diagnosis to AlloSCT, number infused of total nuclear cells, previous transfusions, occurrence of acute and chronic GVHD, infections, acute and late graft rejection and overall survival, between these two conditioning regimens. We analyzed the long-term hematopoietic chimerism by FISH, using a